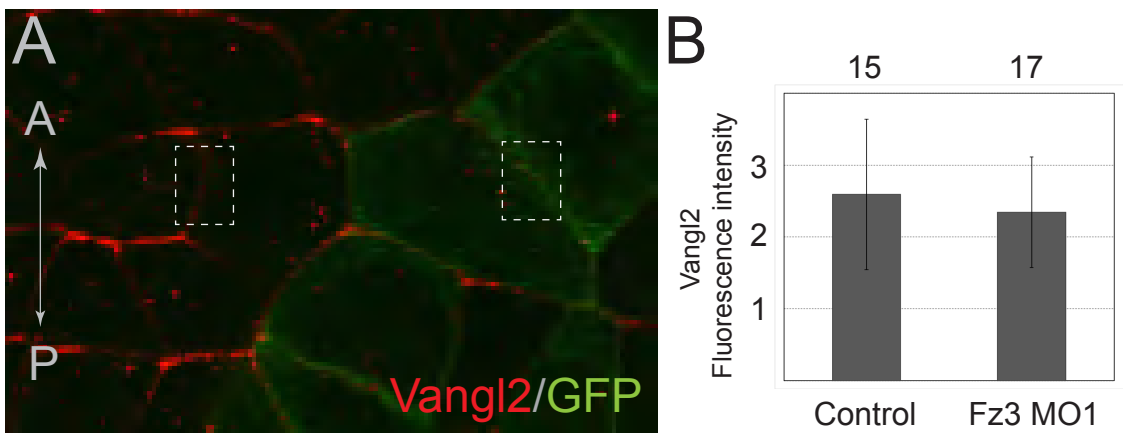

Fig. S1. Fz3 depletion does not affect Vangl2 membrane localization at the mediolateral cell borders. (A) Vangl2-specific fluorescence at the mediolateral cell borders (dashed boxes) was compared between control and Fz3 MO1-injected neural plate cells at stage 16 (from the experiment shown in Fig. 1). GFP (green) is a lineage tracer. Anteroposterior (A-P) axis is indicated. (B) Quantification of mean fluorescence intensity at the mediolateral cell borders in control and Fz3 MO1-injected neuroectoderm cells. Standard deviations are indicated, cell numbers are shown above each bar.

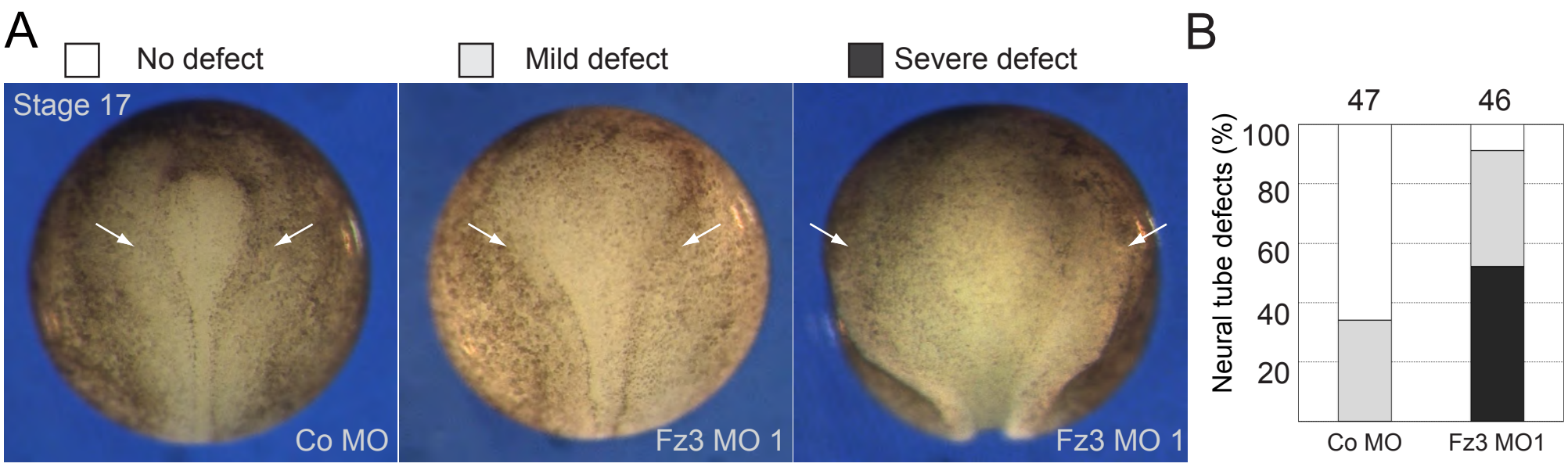

Fig. S2. Depletion of Fz3 causes neural tube closure defects. (A) Two dorsal animal blastomeres were injected with control morpholino (Co MO) (A, left and middle) or Fz3 MO (A, right), 10 ng each. Dorsal view of stage 17 embryos is shown, anterior is at the top. Arrows point to the neural folds. (B) Quantification of the experiments shown in A, showing frequencies of mild (light grey) or severe (dark grey) neural fold defects. The number of scored embryos per group is shown above each bar. Data are representative of three experiments.

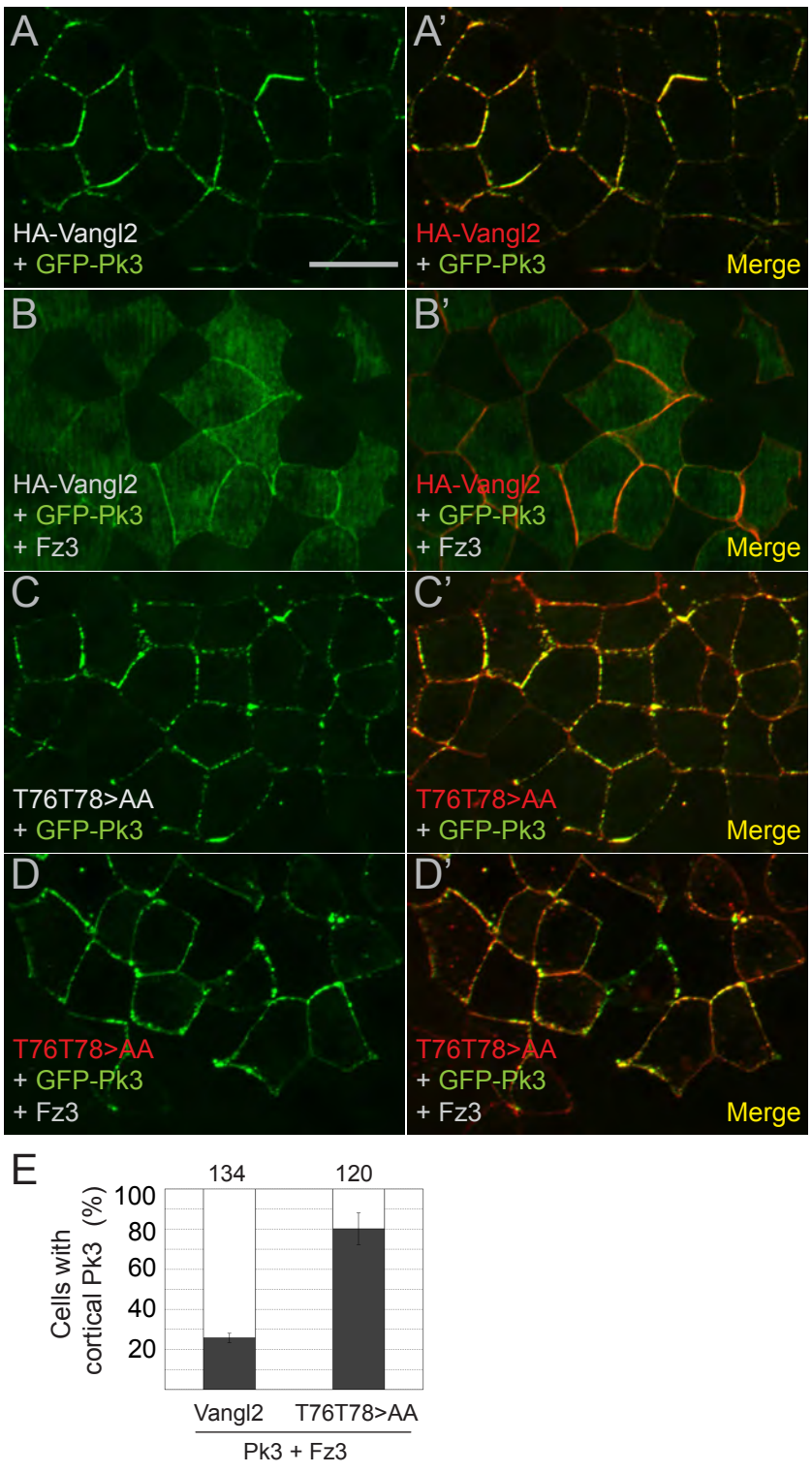

Fig. S3. Vangl2 T76/T78 phosphorylation is necessary for the inhibition of Pk3 cortical localization by Fz3. (A-D') Two dorsal blastomeres of 16 cell embryos were coinjected with mRNAs encoding HA-Vangl2 and T76T78>AA, 100 pg each and GFP-Pk3, 500 pg, with or without Fz3-FLAG mRNA, 400 pg. Vangl2 and Pk3 distribution was analyzed at stage 10.5-11 by anti-HA immunostaining and GFP fluorescence. (A-D') The localization of Pk3 and Vangl2 without (A-A', C-C') or with (B-B', D-D') Fz3 in superficial ectoderm cells. Scale bar, 30 μm. (E) Quantification of data in (B-B') and (D-D') is shown as mean frequencies ± s. d. of the cells containing cortical GFP-Pk3 patches. Numbers of scored cells are shown above each bar; 20 to 50 cells were scored per embryo with five embryos taken for each experimental condition. Data are representative of three experiments.

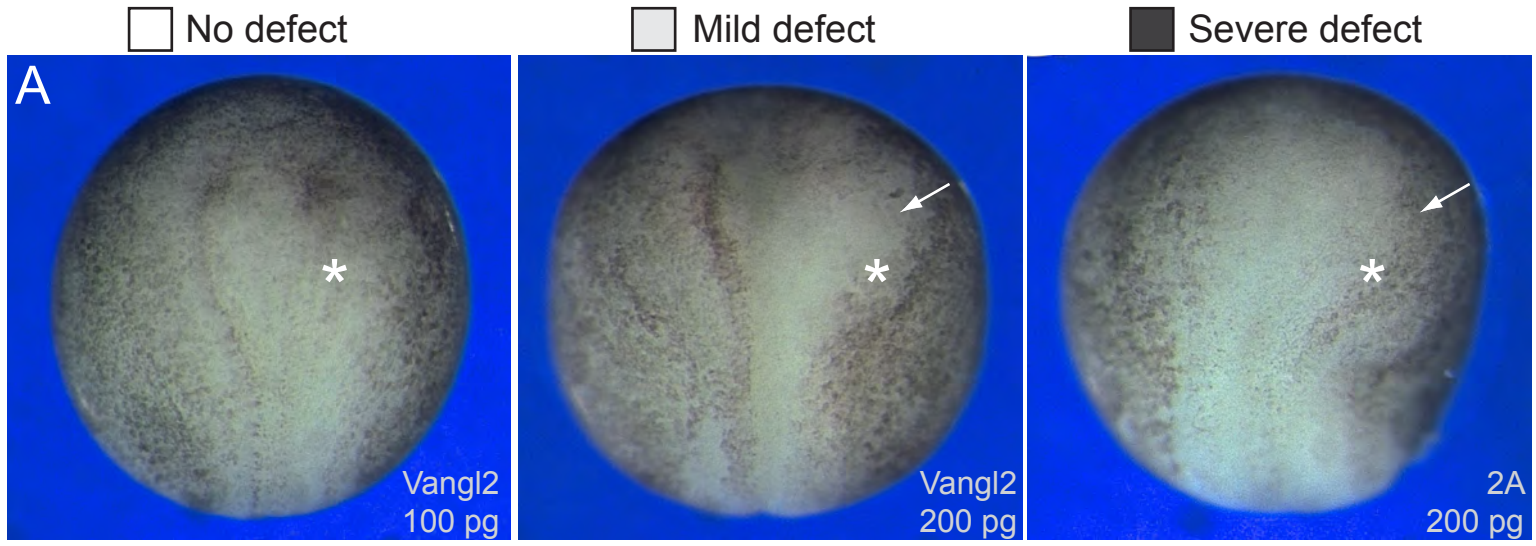

Fig. S4. Expression of Vangl2 T76T78>AA mutant causes neural tube defects. Four-to-eight cell embryos were unilaterally injected with mRNAs encoding Vangl2 or T76T78>AA (2A) constructs, 100, 200 or 300 pg each. (A) Embryo injected with 100 pg of Vangl2 mRNA is indistinguishable from an uninjected embryo (left). Embryo injected with 200 pg of Vangl2 mRNA exhibits mild neural tube defect (middle). Embryo injected with 200 pg of 2A mRNA shows a severe neural tube defect (right). Anterior is at the top. Neural tube defects (arrows) are shown at the injected side (asterisk). (B) Frequencies of mild and severe neural fold defects are shown. Numbers of scored embryos per group are above each bar. Data are representative of two experiments.